

Detection of resistance in populations of pear scab (*Venturia pirini*) and fireblight (*Erwinia amylovora*) in California pear orchards

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Duration of Project: 2 years

Objectives:

1. Determine the presence and level of resistance to DMI and strobilurin fungicides in the *V. pirini* population in California.
2. Determine presence and level of resistance to copper and streptomycin in the *E. amylovora* population in California.

Pear scab and fireblight are two of the most common diseases of pear in California. Both diseases are commonly treated with fungicides and bactericides for control, respectively. In recent years the most common products used against pear scab have been products that attack the pathogen in only one locus. As these materials are used over time, resistance to the products may develop. Growers and pest control advisers (PCAs) have stated that

they have observed a reduced level of control for the DMI and strobilurin fungicides for scab control. At the same time PCAs and growers have also observed a decrease in sensitivity to the products used for fireblight control.

Trifloxystrobin (Flint) is active against a wide array of pathogens including the pear scab pathogen *V. Pirini*. This product inhibits mitochondrial respiration by blocking electron transfer at the ubiquinol-oxidation center (Qo-site) of the bc1-enzyme complex (complex III). Based on evidence from a number of pathogens, trifloxystrobin, kresoxim-methyl, azoxystrobin and famoxadone belong to the same cross-resistance group, the Qo-site inhibitors (QoIs).

The baseline sensitivity of *V. pirini* towards trifloxystrobin has not been previously established. However, the mean baseline sensitivity towards *V. inaequalis*, a closely related species causing apple scab, was found to be .0035 ppm with a rather narrow range of sensitivity (factor of up to 16 between the most and the least sensitive isolates). In our study, we found one orchard that had not been exposed to any strobilurin fungicide and therefore our baseline sensitivity comes from that orchard. However, at present we are still running those isolates to see what the range in sensitivity is. We can though, make the statement that nearly all those isolates have EC50 values close to zero. In our tests the lowest concentration was 0.032 which is too high to pick up those isolates that would fall into the 0.0035 range. However, the group of isolates that we are calling wild type did not grow at 0.032 ppm which means they have EC50's of less than that. We won't know until we rerun that group whether all fall into true baseline numbers. This is currently being done and an updated addendum to the annual report will be submitted later.

Results:

Isolates of *V. pirini* were collected from California pear orchards in 2006 and were taken to UC Davis and/or UC Riverside to be mass cultured and maintained on appropriate media prior to being tested on poison agar plates for their reaction to various fungicide and bactericide products. In the case of screening for resistance to Flint (trifloxystrobin), the fungicide was incorporated into artificial media and each of 502 isolates was streaked onto the plate. Each isolate was replicated at least three times and the test repeated at least one time. Data analysis was conducted for determination the EC50 in ppm against trifloxystrobin and for determination of significant differences among isolates for individual products.

In order to assess the presence of resistance in the *V. pirini* population in California pear orchards we used the spiral plating technique as described by Adaskaveg et al. Fresh leaf

and fruit samples were sent to the lab and the pathogen was isolated from lesions onto potato dextrose agar amended with tetracycline (PDA-tet). Cultures were then stored at 4 C until processed for assessment of resistance. Petri dishes containing PDA were placed on the spiral plating instrument and trifloxystrobin was incorporated into the media in a spiral pattern whereby the highest concentration of fungicide was placed in the middle of the plate and as the needle which incorporated the fungicide spiraled toward the outside of the plate the fungicide became less concentrated (Fig1). The fungus was then applied to the media using a sterile plastic spatula (Fig 2) with the experimental design having two replications per plate.

Isolates were identified as to the location from which they were collected, including pear variety, orchard, and location of orchard in the county. A history of use for the product class being put together but has not been completed yet.

Results of this study are not fully complete but if we assume a similar baseline sensitivity in the *V. pirini* population as has been shown for the *V. inaequalis* population then many of the orchards assayed showed some degree of resistance. Only in two orchards did the isolates show close to normal mean sensitivity, #'s 38 and 8, (Fig 3), while nearly all orchards contained isolates that could be considered as wild type. Mean isolate populations from the remaining orchards ranged in EC50 values from 0.03 to 0.63.

Isolates from Mendocino County ranged in sensitivity from 0 to 2.83 ppm (Fig 6). Two hundred and five out of 279 Mendocino County isolates showed a sensitivity of less than 0.032. Another way to look at this is that 73.5% of the isolates could still be considered as being representative of wild type isolates or still very sensitive to trifloxystrobin and therefore easily controlled by the product. The other 26.5% showed varying degrees of sensitivity with some showing a high level of resistance (Fig 6).

One hundred and eighteen isolates were sampled from Lake County with 87 having EC50 values less than 0.032. Thus 73.7% of the isolates are considered to be wild type. The remaining isolates ranged in sensitivity from .05-2.85 (Fig 9).

One hundred and thirty four isolates were collected from other locations. Of these isolates, 85 or 63.4% had EC50's of less than .005 and therefore could be considered as wild type isolates. The remaining isolates ranged in EC50 from .05- 1.35 (Fig.7).

Variety of pear was significant in terms of disease. However, because Bartlett is the most widely planted variety it stands to reason that we would see more disease and therefore potentially more resistance show up in the isolates attacking that variety (Fig 8).

When all the isolates from all counties were analyzed (Fig. 5), we see that most isolates

were sensitive to trifloystrobin. More isolates from Mendocino County showed resistance to trifloxystrobin but there was a higher level of resistance in Lake County orchards.

Conclusions:

Resistance to trifloxystrobin has been shown to occur in the *V. pirini* population in 2 major pear production areas in California. The level of resistance is considered high in some orchards and regions and probably could account for reduced degree of control of pear scab over the past 2 years. Resistance management in the *V. pirini* population is still an option and should allow the strobilurin fungicides to be used effectively. Resistance management should be practiced with all the knowledge available. Some suggestions for continued use of these fungicides are to follow the FRAC guidelines: 1. Use strobilurin fungicides only as preventative, 2. Use minimum number of applications in a given year, 3. Use only according to the manufacturers recommendations, 4. Use Qol's as a single block then switch to different chemistry.

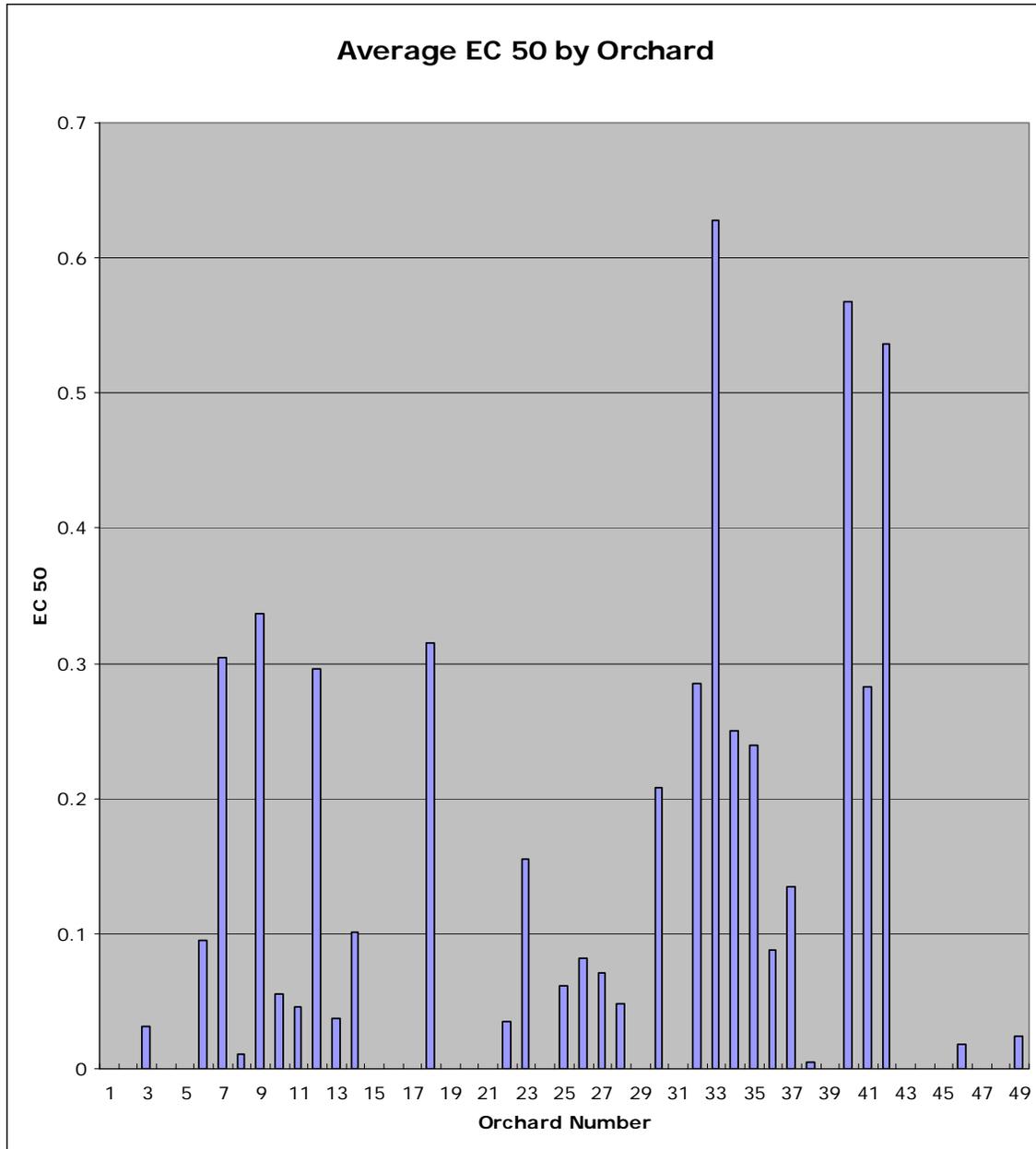


Fig. 4. Orchards from which *V. pirini* was sampled and mean EC50 against trifloxystrobin (Flint) for each orchard.

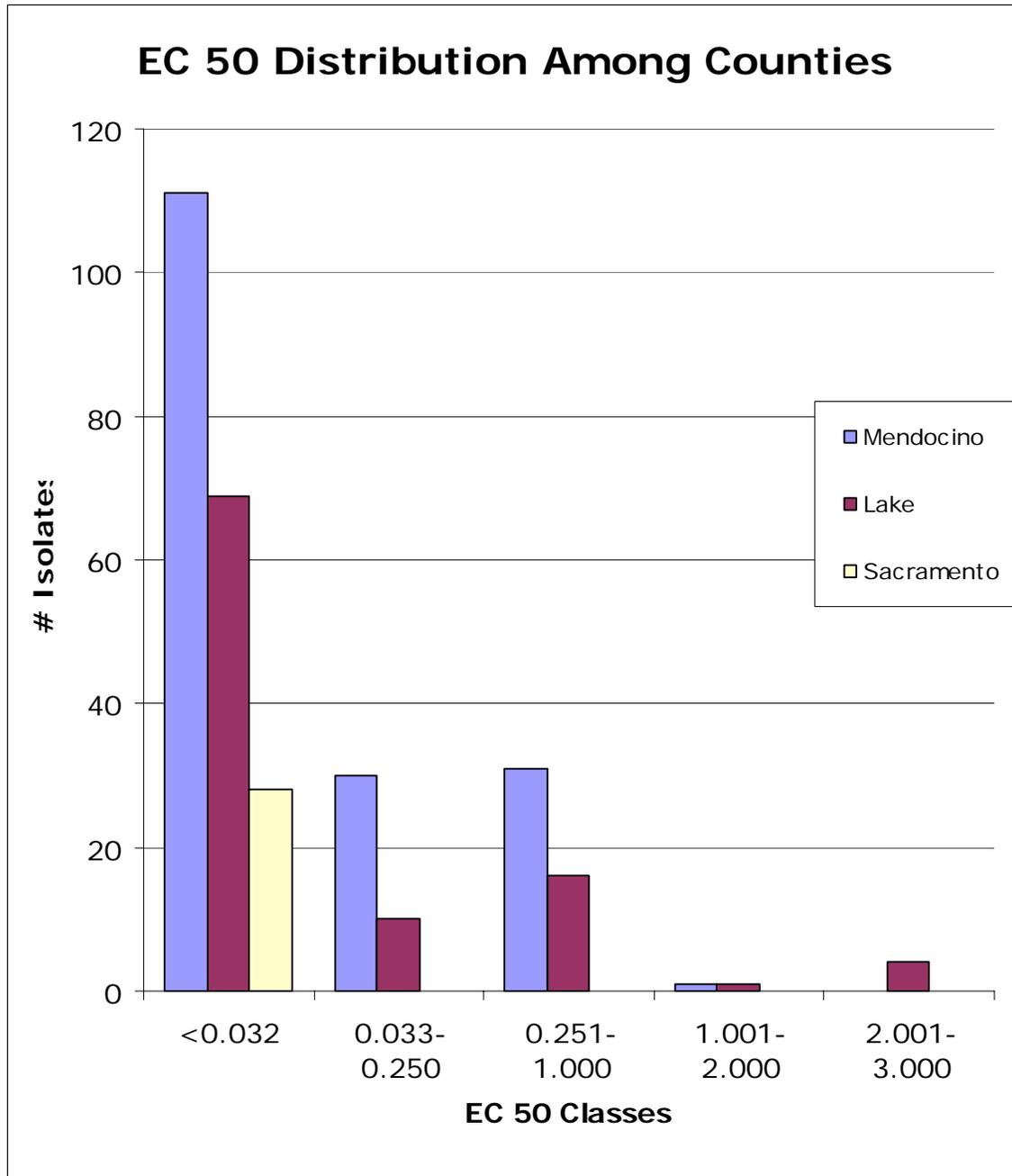


Fig. 5. Sensitivity (EC₅₀) of *V. pirini* isolates from 3 pear production counties to trifloxystrobin. Isolates with EC₅₀ values less than 0.032 are considered to be highly sensitive. This group (<0.032) showed no growth at this concentration and is being rerun to develop the range of sensitivity at lower concentrations. Isolates with EC₅₀ values greater than this figure are considered to be resistant to trifloxystrobin with those growing at 2-3 ppm being the most resistant.

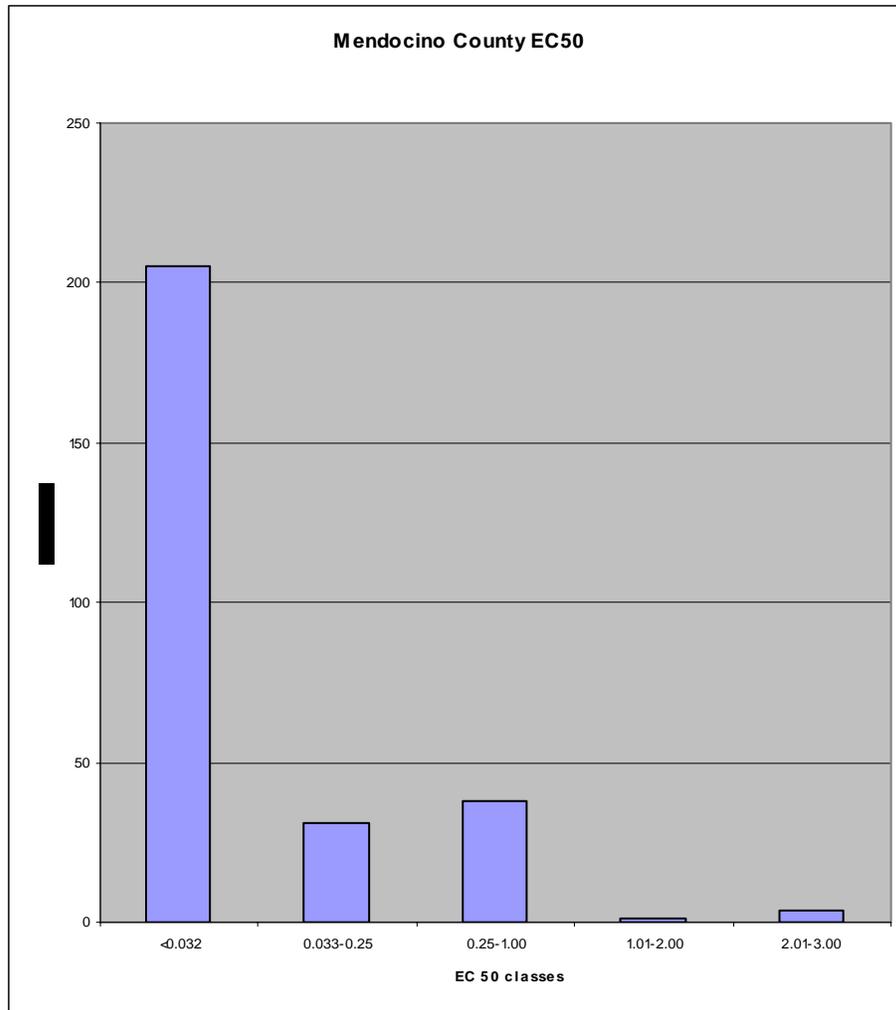


Fig. 6. Sensitivity of Mendocino County *V. pirini* isolates towards trifloxystrobin. Isolates with sensitivity less than 0.032 did not grow at this concentration and are considered to be highly sensitive to trifloxystrobin while those with EC50's greater than 0.032 are considered to be resistant.

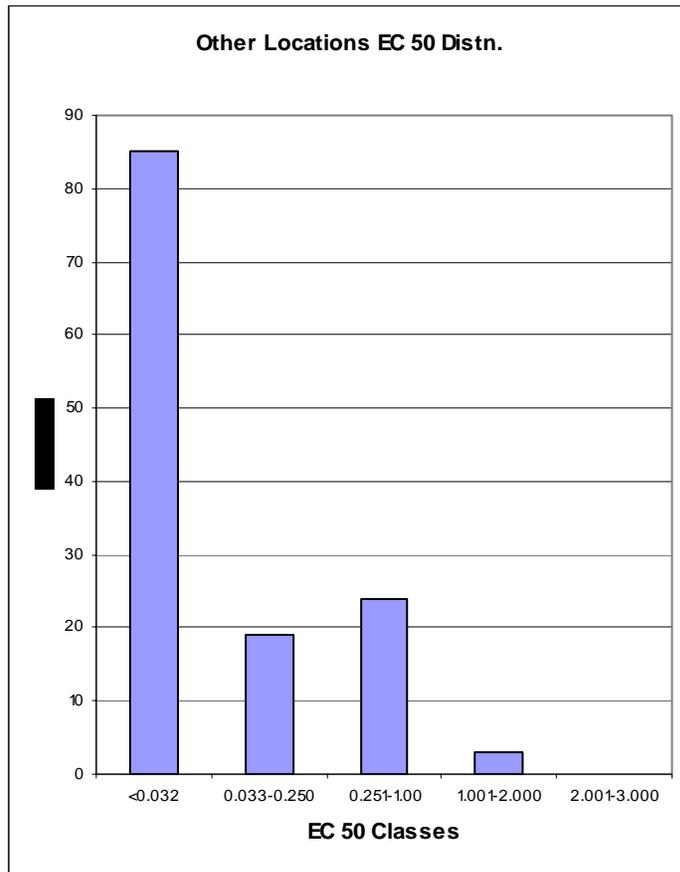


Fig. 7. Sensitivity of *V. pirini* isolates collected from Yuba County and other locations in California. Isolates with EC50's of less than 0.032 are considered to be wild type isolates with no resistance while those with EC50's of greater than 0.032 are considered to be resistant.

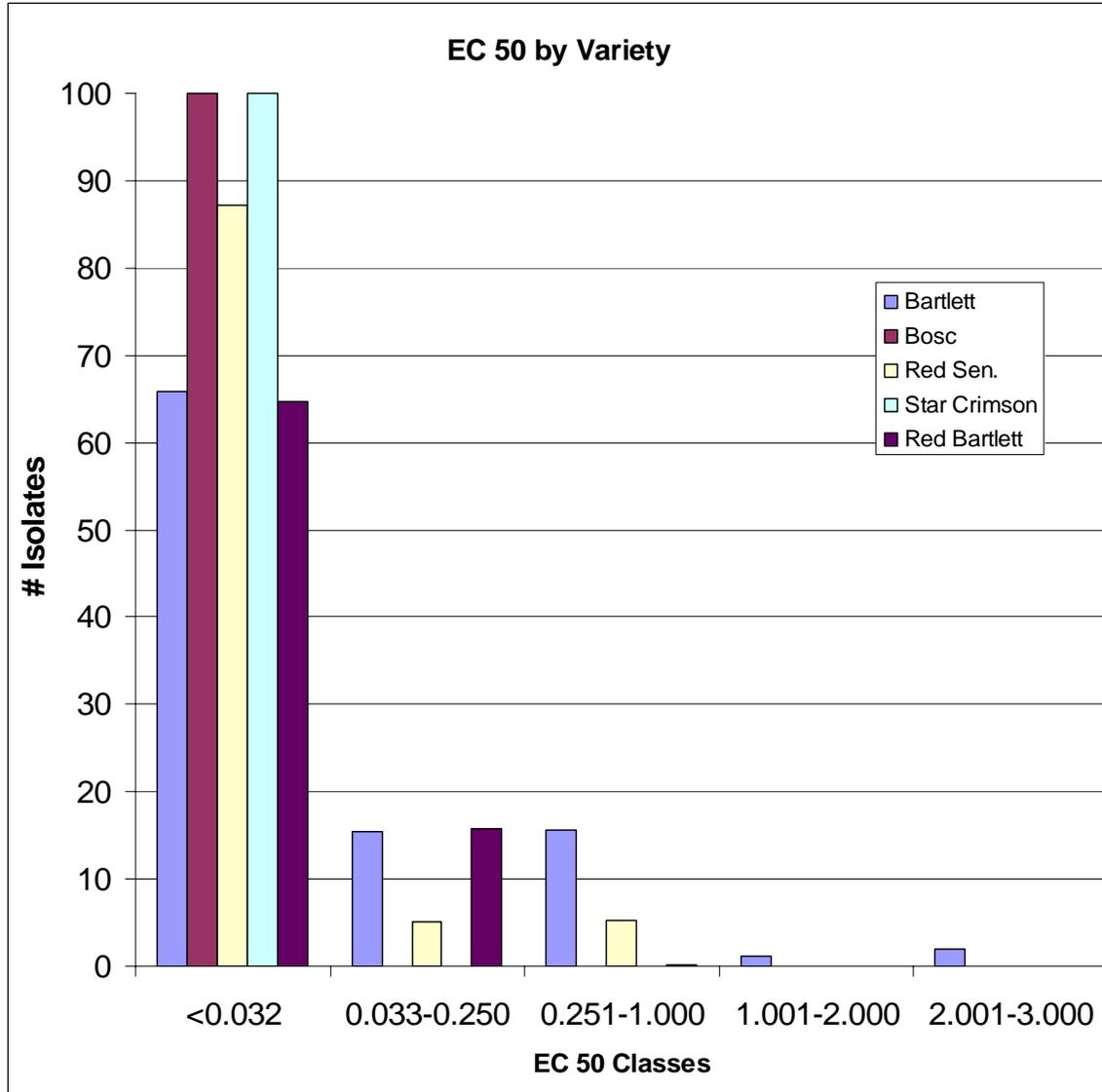


Fig. 8. Sensitivity of *V. pirini* isolates collected from different pear varieties. Isolates with EC50's of less than 0.032 are considered to be wild type isolates with no resistance while those with EC50's of greater than 0.032 are considered to be resistant.

Fig. 9. Sensitivity of Lake County *V. pirini* isolates toward trifloxystrobin. Isolates with sensitivity less than 0.032 did not grow at this concentration and are considered to be highly sensitive to trifloxystrobin while those with EC50's greater than 0.032 are considered to be resistant.

